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Animal Model of Human Medulloblastoma: Clinical, Magnetic Resonance Imaging, and Histopathological Findings After Intra-Cisternal Injection of MHH-MED-1 Cells Into Nude Rats

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To establish an animal model of human medulloblastoma, we have injected human MHH-MED-1 cells into the cisterna magna of nude rats. Tumors grew in 3 out of 4 animals injected with 10^6 medulloblastoma cells. Affected animals showed little or no weight gain and eventually lost weight but did not develop obvious neurological symptoms until the end of observation on day 31 after inoculation. At this time, magnetic resonance imaging (MRI) in tumor-bearing rats revealed contrast enhancement in the region of the fourth ventricle and the cisterna magna. Neuropathological examination demonstrated corresponding leptomeningeal growth in the cisterna magna invading the medulla oblongata, and tumor growth within the fourth ventricle invading the pons. The tumors basically showed the same immunostaining pattern as MHH-MED-1 cells in vitro expressing neuron-specific enolase (NSE) and vimentin, but no neurofilaments (NFs), synapthophysin, or glial fibrillary acidic protein (GFAP). No tumor grew in the fourth animal, which had a normal weight gain and no alteration on MRI. In conclusion (1) the intrathecally injected human medulloblastoma cells spread similar to medulloblastomas in patients, (2) tumor growth is readily detected by MRI, (3) the new animal model is a suitable tool for further experimental research including intrathecal therapeutic studies. Med. Pediatr. Oncol. 29:92–97, 1997.

Key words: medulloblastoma; cell line; xenograft; nude rat; leptomeningeal dissemination

INTRODUCTION

In spite of all the progress made in the treatment of medulloblastoma over the last 20 years some 50% of patients still die of recurrent disease. The surviving patients often suffer from late effects of therapy [1–3]. Appropriate animal models of human disease in addition to in vitro studies of established medulloblastoma cell lines might yield important pathophysiological findings. They may be the basis of new, especially intrathecal, therapeutic strategies. Experiments with nude mice have mainly studied growth patterns and the effects of systemic chemotherapy after subcutaneous, intracerebral, or intracisternal inoculation of medulloblastoma cells [4–7]. Because of its small size the nude mouse model was not suitable for experimental intrathecal therapy. A nude rat model amenable to experimental intrathecal therapy has recently been reported [5]. It makes use of lumbar intrathecal injection of human medulloblastoma cells through a permanently implanted catheter, giving rise to diffuse leptomeningeal metastasis thus simulating advanced stages of the human disease. To imitate an earlier clinical situation with localized posterior fossa growth (and eventual leptomeningeal dissemination) we inoculated athymic rats cisternally with human MHH-MED-1 medulloblastoma cells. The animals were followed clinically for 31 days. Tumor growth was finally evaluated by MRI and histologically as reported herewith.

MATERIALS AND METHODS

MHH-MED-1 Cell Line

The MHH-MED-1 cell line has been generated by one of us (T.P.) from tumor cells spread into the cerebrospinal fluid of a 10-year-old boy with relapsing medulloblastoma [8]. Histological diagnosis according to the WHO classification of brain tumors was medulloblastoma (primitive neuroectodermal tumor of cerebellum).

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TABLE I. In Vivo and Neuropathological Findings 31 Days After Cisternal Inoculation of MHH-MED-1 Cells Into Nude Rats

Rat no.	Weight gain (%)[a]	MRI[b]	Neuropathological findings: Tumor growth[c] Cisterna magna/4th ventricle	Tumor growth[c] Medulla obl./pons	Immunohistochemistry[d] NSE	VIM	GFAP	SYN	NFs
1	−36	+	+/+	+/+	+	+	(+)	−	−
2	−11	+	+/+	+/+	+	+	(+)	−	−
3	−4	+	+/+	+/+	+	+	(+)	−	−
4	+36	−/−	−/−	−/−					

[a]Day 31 compared with day 0.
[b]+, contrast enhancement in the region of the 4th ventricle and the cisterna magna; −, no enhancement.
[c]+, tumor growth; −, no tumor growth
[d](+), single cells; +, >80% of cells; NSE, neuron specific enolase; VIM, vimentin; GFAP, glial fibrillary acidic protein; SYN, synaptophysin; NFs, neurofilaments.

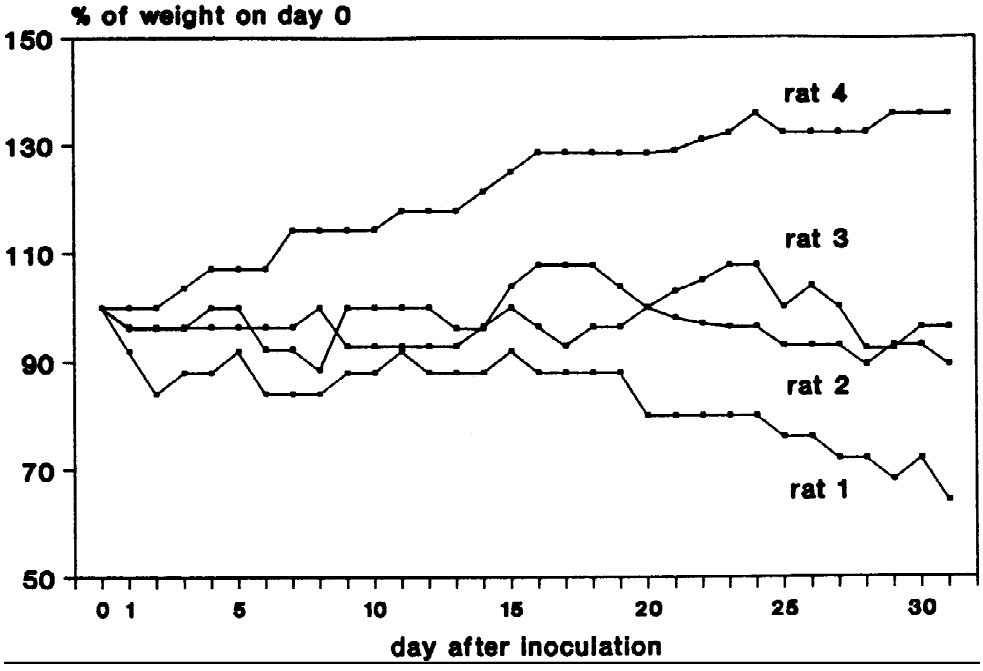

Fig. 1. Weight course of rats 1–4 after cisternal inoculation of 10^6 MHH-MED-1 cells.

Immunocytochemistry showed expression of vimentin and neuron-specific enolase (NSE), but not of synaptophysin, neurofilaments (NF), or glial fibrillary acidic protein (GFAP). The tumor cells in cerebrospinal fluid were sedimented by centrifugation (800*g*) and resuspended in DMEM, high glucose formulation (Gibco, Eggenstein, Germany) supplemented with 2 mM L-glutamine and 10% heat-inactivated human serum. The cultures were split every 5 days in a ratio of about 1:2 and have now been continued for more than 3 years. The cells grow in suspension in spherical aggregates. They show undifferentiated morphology and like the original tumor express vimentin and NSE but neither NFs nor GFAP. Karyotype is near diploid. The cell line does not grow when cells are injected subcutaneously into nude mice [8].

Inoculation of Tumor Cells and Clinical Observation

Four female Rowett nude rats weighing 120–140 g were obtained from the Zentrale Tierzuchtanstalt in Hannover, housed under normal conditions, and fed ad libitum. As previously described [9] they were inoculated with tumor cells under anesthesia with 62.5 mg/kg ketanest (Ketavet™, Parke Davis, Berlin, Germany) and 2.87 mg/kg xylazin (Rompun™, Bayer, Leverkusen, Germany). Briefly, after microsurgical exposure of the atlantooccipital membrane, 50 μl of cerebrospinal fluid was aspirated through a 27 G needle and replaced by 50 μl of DMEM containing 10^6 MHH-MED-1 medulloblastoma cells (passage 50). The wound was sutured with 3-0 polyglactin 910, and 4-0 silk. Animals were followed

with daily weights and neurological examinations. On day 31 after inoculation all animals obtained MRI scans and were subsequently sacrificed with an overdose of ketanest and xylazin (see above).

MRI

Animals were anesthetized with a halothane/N_20/O_2 open system. Imaging was performed using a special resonator (inner diameter 7.5 cm) in a 1.5 T whole-body Siemens (Knoxville, TN) Magnetom system (maximum gradient field strength 10 mT/m) as published previously [10]. A 6×6 cm^2 field of view with a 256×256 matrix was achieved with a read-out time of 11.5 ms. Spin-echo sequence parameters were adjusted to 3 mm slices with an intersclice gap of 1.5 mm. Repetition time (TR) was 500 ms, and echo time (TE) 28 ms. As a contrast material 0.5 mmol/kg Gd-DTPA (Magnevist™, Schering, Berlin, Germany) was injected via the tail vein with a concentration of 0.25 mmol/ml in normal saline.

Neuropathological Evaluation

The brain and spinal cord were prepared together with the meninges and fixed in formalin as described previously [9]. Then the brain was cut into 4 coronal sections at the levels of the optic chiasm, the exit zone of the 3rd cranial nerve, the midpontine region (through the fourth ventricle), and the medulla oblongata oblongata (through the cisterna magna). The spinal cord was cut into 3 axial sections at the cervical, thoracic, and lumbar level. The resulting tissue blocks were embedded in paraffin. Standard 6-μm sections were mounted on poly-L-lysine-coated slides, and stained with hematoxylin and eosin. For immunohistological analysis, monoclonal antibodies against vimentin, synaptophysin (Boehringer, Mannheim, Germany), and the 68, 160, and 200 kd NFs (Amersham, Braunschweig, Germany) and rabbit antisera against GFAP (Serva, Heidelberg, Germany), and NSE (Dianova-Immunotech, Hamburg, Germany) were employed. Antibody-binding was visualized by the avidin-biotin (ABC)-peroxidase method [11].

RESULTS

Clinical Findings

After intracisternal inoculation of tumor cells Rat 1, 2, and 3 did not normally increase in weight and eventually lost weight starting around day 20 in Rat 1 and 2, and on day 25 in Rat 3 (Table I, Fig. 1). None of these rats developed obvious neurological symptoms until the end of observation on day 31. Rat 4 had a normal weight gain.

MRI Findings

On day 31 after inoculation of tumor cells, Gd-DTPA enhanced T_1-weighted images in Rat 1 revealed intense contrast enhancement extending laterally from the cisterna magna and the fourth ventricle and invading the left dorsal pons and medulla oblongata (Fig. 2A). Also in this rat and in Rat 2 and Rat 3, contrast enhancement was clearly visible in the midline corresponding to the fourth ventricle and the cisterna magna (Fig. 2A–C). On the precontrast T_1-weighted images no signal abnormalities were seen except for Rat 1, which revealed a mild space-occupying lesion at the site of Gd-enhancement (not shown). No enhancing lesion or other abnormalities were seen in Rat 4 (Fig. 2D).

Neuropathological Findings

Macroscopic examination showed thickening of the pial membrane within the cisterna magna and some whitish material within the fourth ventricle of Rats 1, 2, and 3. Histology in these rats demonstrated tumor growth in the fourth ventricle and in the cisterna magna with invasion of the cerebellum, the pons and the medulla oblongata, respectively (Table I, Fig. 3). No other sites of the neuroaxis were affected. Immunohistochemistry showed NSE and vimentin in the majority of cells and GFAP in a few single cells with long processes while staining for NFs and synaptophysin was negative (Fig. 3). Tumor growth in Rat 1, 2, and 3 corresponded to the pathological contrast enhancement seen on MRI and was most extensive in Rat 1 (Fig. 2A–C). No tumor was found in Rat 4.

DISCUSSION

The study of human medulloblastoma cell lines in vitro and in appropriate animal models should provide new therapeutic insights into the human disease. Ironically, the TE-671 cell line [12] first and most widely used for in vitro drug testing [3,4] turned to be a subclone of a rhabdomyosarcoma cell line [13]. Continous medulloblastoma cell lines currently available for experimental studies, are lines D283, D341, D384, D425, and D458 [5], lines ONS-76, and ONS-81 [14], Daoy [15], and 4 lines called MHH-MED-1 to -4, which were established and characterized by one of us [8]. The D (Duke) lines and the MHH-MED-1 line grow in suspension, while the Daoy and ONS lines grow as adherent monolayer. The D and ONS lines show neuronal, Daoy some glial differentiation. MHH-MED-1 in vitro coexpresses vimentin and NSE, but no NFs, syaptophysin or GFAP representing an immature phenotype. As none of the known medulloblastoma cell line expresses GFAP, our finding of GFAP positivity in single cells within the MHH-MED-1 posterior fossa tumor is questionable and might be explained by the presence of rodent ‘‘contaminating’’ reactive astrocytes in the xenografted tumor [5]. This hypothesis is supported by the finding that these GFAP

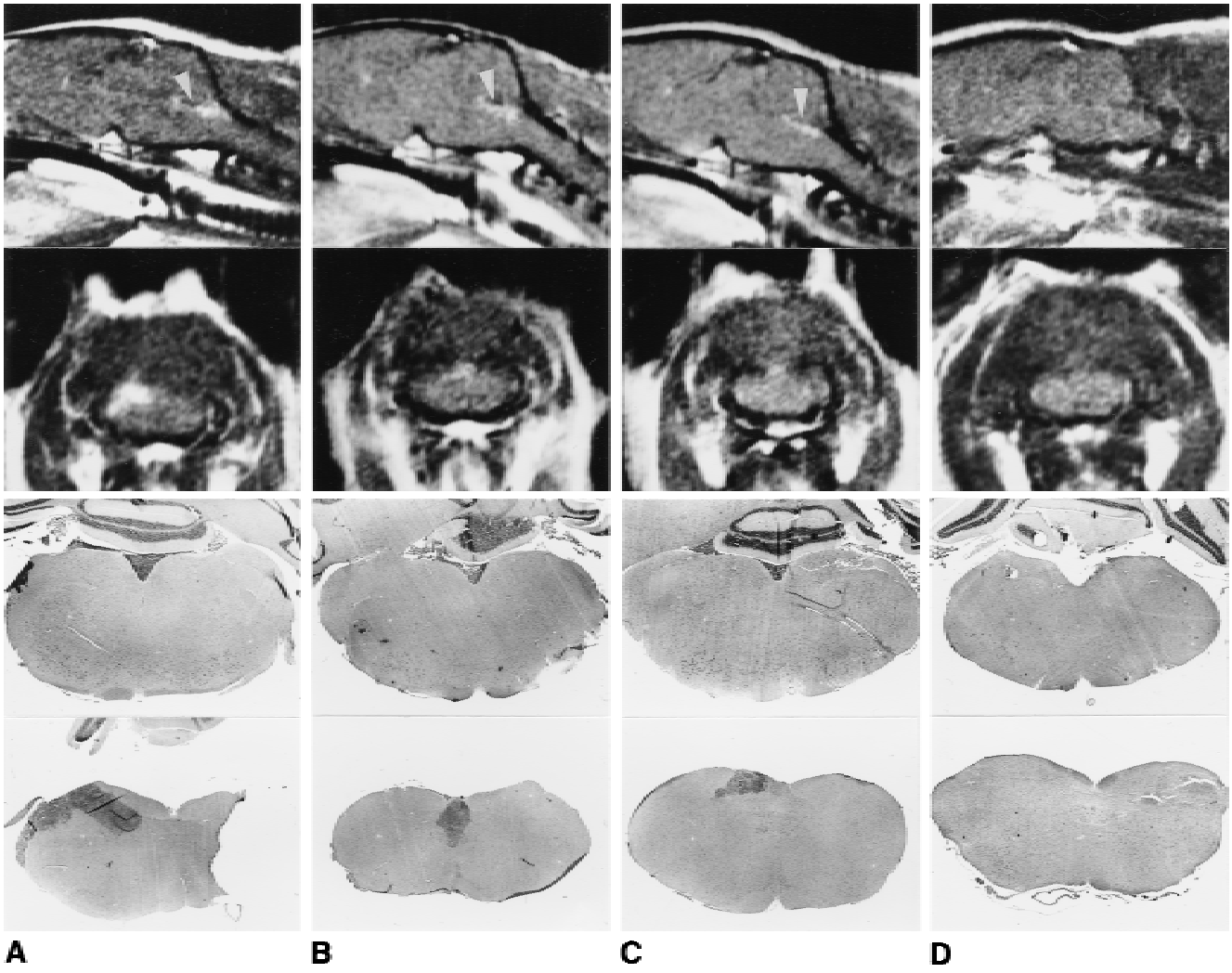

Fig. 2. Mediosagittal (1st row) and coronal (2nd row) T_1-weighted Gadolinium enhanced MRI, corresponding H.E. stained 6 μm coronal sections through the pons and the fourth ventricle (3rd row), through the medulla oblongata and the cisterna magna (4th row). **(A)** Rat 1, **(B)** Rat 2, **(C)** Rat 3, **(D)** Rat 4.

positive cells had long cytoplasmic processes, which is a characteristic feature of reactive astrocytes.

Subcutaneous and intracerebral xenografts in nude mice have been used for experimental intravenous chemotherapy with alkylating agents [4] and supported the clinical use of these drugs [16,17]. The rationale for intrathecal therapy of localized or subarachnoidal spreading medulloblastoma with methotrexate, thiotepa, or other substances including ^{131}I-labelled monoclonal antibodies against tumor antigens or allogeneic LAK cells [2,16,18,19] has not been experimentally elaborated. This could be accomplished by using our nude rat model or the animal model of Friedman and coworkers [5]. One needs to keep in mind, however, that both models represent different stages of the human disease and use different approaches to the CSF.

MRI evaluation of experimental medulloblastoma has not been reported before. Our findings indicate that it is sensitive enough to allow longitudinal evaluation of tumor growth in the same animal. This should give further insight into therapeutic effects in vivo and with the addition of new data for statistic analysis also might spare animals in therapeutic experiments. Meanwhile we have reproduced this experimental model in another 9 animals. Eight of these animals developed medulloblastomas with leptomeningeal spread until day 50. Therapeutic studies are currently performed.

CONCLUSION

In conclusion, the intrathecally xenografted human MHH-MED-1 medulloblastoma cells spread similar to medulloblastomas in patients, and tumor growth is readily detected by MRI. Therefore, this new animal model is a suitable tool for further experimental research including intrathecal therapeutic studies.

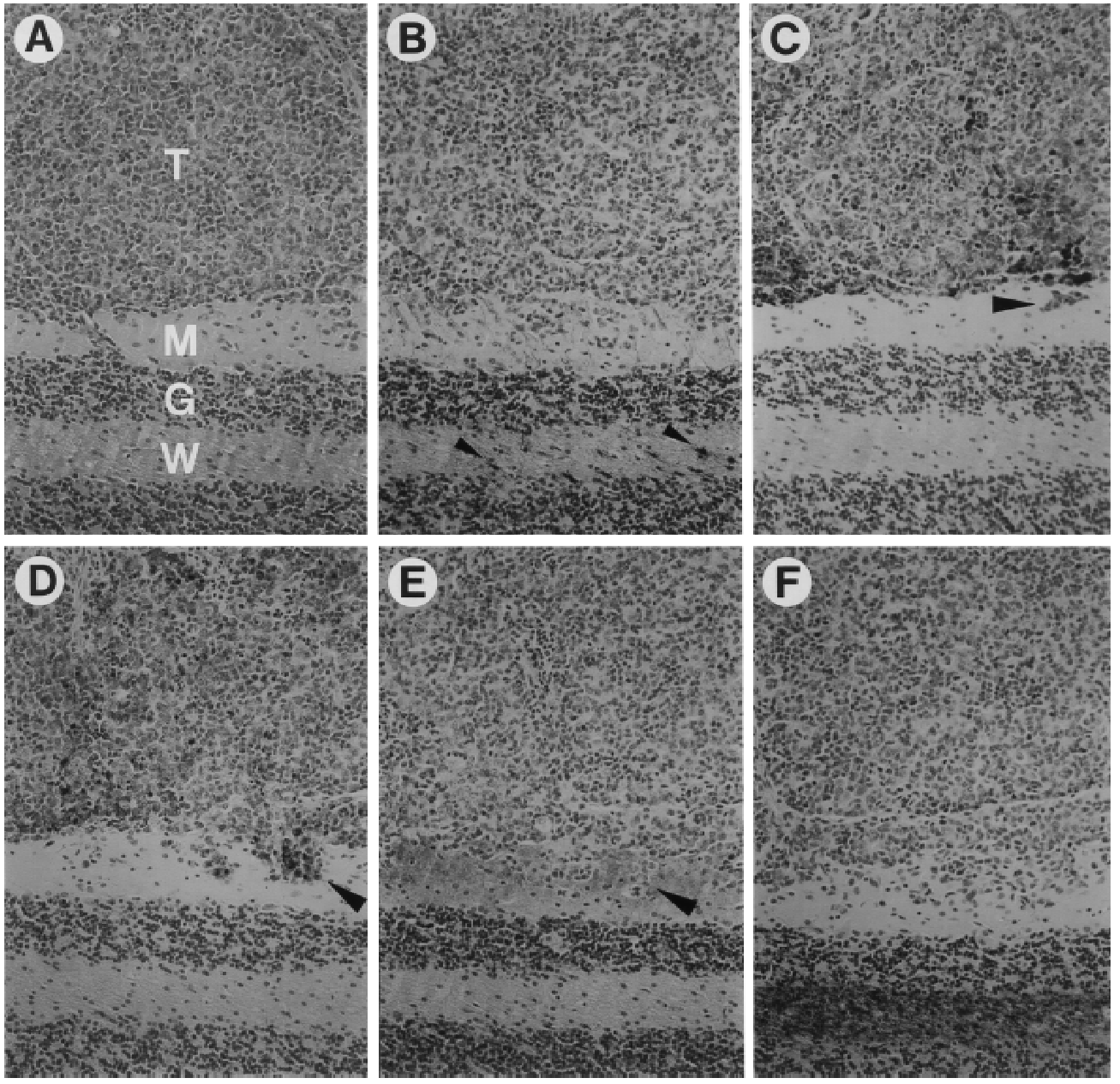

Fig. 3. Immunohistochemistry of MHH-MED-1 xenotransplanted tumor invading the rat cerebellum. **A:** H.E. stain; T, tumor; M, molecular layer of the cerebellum; G, granular layer; W, cerebellar white matter. **B:** Anti-GFAP immonoperoxidase reaction; small arrowheads, reactive astrocytes in the cerebellum, the tumor is GFAP-negative. **C:** Anti-NSE, positive tumor cells; arrowhead, infiltration of NSE-positive tumor cells into the molecular layer of the rat cerebellum. **D:** Anti-vimentin; arrowhead, vimentin-positive infiltrating tumor cells. **E:** Anti-synaptophysin; arrowhead, synaptophysin-negative tumor cells infiltrate the synaptophysin-positive molecular layer. **F:** Anti-neurofilaments; tumor cells are negative, cerebellar white matter positive. Magnification ×100.

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